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Oxygen Effects on Photosynthesis and ^{14}C Metabolism in Desert Plants

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ABSTRACT

The effect of 1% and 21% O_2 upon $^{14}CO_2$ assimilation by desert plants exposed for 10 to 90 seconds has been studied. The plants studied can be divided into three groups with respect to O_2. The C_3 plants display the usual Warburg effect. No changes could be observed in the intensity of photosynthesis as a function of O_2 content in another group of plants (showing signs of Crassulacean acid metabolism). In still another group of plants (C_4 plants) the stimulating effect of O_2 on photosynthesis could be detected. In C_3 plants, O_2 inhibits the processing of carbon through the Calvin cycle intermediates. The involvement of carbon in the glycolate pathway fails to explain completely the inhibiting effect of O_2 on photosynthesis. It is assumed that O_2 inhibits the enzymes of the Calvin cycle. In C_4 plants O_2 stimulates the incorporation of ^{14}C into malate and aspartate. The incorporation of ^{14}C into the intermediates of the Calvin cycle in C_4 plants is inhibited much like that in typical C_3 plants.

Research on the effects of O_2 on photosynthesis and metabolism of desert plants is a continuation of the investigative project concerned with the ecology, physiology, and biochemistry of photosynthesis developed by the Laboratory of Photosynthesis of the Komarov Botanical Institute particularly in the field experiments performed in the hot desert of the Southeastern Kara-Kum (23). The results obtained in previous studies have been published (29, 34). Briefly they may be summarized as follows:

Estimation of the intensity of the true (potential) photosynthesis (at 1% CO_2) performed by the $^{14}CO_2$ method (31) in 30 edificator plant species compared with the assessment of the intensity of net photosynthesis by the conductometric technique (30) revealed large interspecies differences. For instance, the highest average intensity of the true and net photosynthesis over the growing period has been reported for the grass *Aristida karelinii* (C_4), the semishrub *Smirnowia turkestana* (C_3), and the herbaceous ephemeroid *Ferula litwinowiana* (C_3). Low rates of true and net photosynthesis are typical of long growing aphyllous shrubs (*Haloxylon aphyllum, H. persicum* and other species). In all species studied there was no midday depression of photosynthesis and evolution of CO_2. Seasonal variations of photosynthesis are manifested in a decrease in the intensity with onset of a dry and hot period, which extends until autumn in the long vegetating species.

Many species of the Kara-Kum desert plants are capable of assimilating CO_2 over a wide range of temperatures. True photosynthesis is active at temperatures from −5 to +55 C, the net photosynthesis up to 40 to 45 C. The temperature optimum for photosynthesis in the majority of the species is 25 to 30 C. The long growing species (genus *Haloxylon* and others) show a very wide range (up to 20 C) of temperatures optimum for photosynthesis. Saturation of photosynthesis by light in the majority of the species occurs at 50 to 70% of full solar radiation. At elevated CO_2 concentrations in a number of species, photosynthesis does not reach light saturation (29, 33, 34).

With respect to their anatomical structure (24, 27, 28), the kinetics of metabolism (9), the efficiency of the isotope discrimination of carbon, and on the basis of other characteristics (34) the Kara-Kum plants may be divided into three groups: (*a*) C_3 plants representing the prevailing majority of the Kara-Kum flora species; (*b*) C_4 plants, of which only four species have been detected in the Kara-Kum flora (*Aristida karelinii, A. pennata, Atriplex dimorphostegia*, and the exogenous plant *Cynodon dactylon*; (*c*) the plants putatively referred to the CAM group. Among these in the Kara-Kum one finds the aphyllous shrubs widely distributed (*Haloxylon aphyllum, H. persicum*) and shrubs with cylindrical leaves (*Salsola richteri*) of the family Chenopodiaceae. These display a peculiar anatomical structure distinct from the typical Kranz-type and succulent species (well developed water-bearing tissue). With respect to carbon isotope discrimination, $\delta^{13}C$, these species are close to C_4 plants. In contrast to the latter they display diurnal variations of their pH and organic acids, these variations being typical of CAM plants. At night these plants assimilate CO_2 only slightly under our conditions.

A study of photorespiration based on $^{14}CO_2$ evolution (35) in 26 edificator species has revealed that the values of photorespiration are not large and, as a rule, do not exceed 5% of the assimilated carbon during photosynthesis (11). Moreover, values of photorespiration did not correlate with the intensity of photosynthesis. With a decrease in the O_2 content down to 1% the evolution of $^{14}CO_2$ was reduced in all of the species studied (both C_3 and C_4). These studies concluded that there are complex interrelationships between photosynthesis and photorespiration and that O_2 affects directly the mechanism of photosynthesis (12).

The present investigation was aimed to estimate the effect of O_2 on $^{14}CO_2$ assimilation during pulse (seconds) exposures under conditions which approach those typical of normal rate of photosynthesis.

MATERIALS AND METHODS

Edificator species belonging to various ecological groups have been chosen in natural desert conditions for the present investigation. Among C_3 plants long vegetating shrubs such as *Smirnowia turkestana, Ammodendron conollyi* of the Leguminosae family and others have been studied. *Haloxylon aphyllum, H. persicum, Salsola richteri* were chosen as representatives of plants with succulent structural features in their assimilating organs. Of the C_4 plants the following species were studied: *Aristida karelinii, A. pennata, Atriplex dimorphostegia*, and the exogenous plant *Cynodon dactylon*.

Photosynthesis and ^{14}C metabolism were monitored by supplying $^{14}CO_2$ (0.04 and 0.5%) (32). In some experiments kinetic measurements were performed at exposures of 10 to 90 sec. Use of pulse exposures favored assessment of the actual rate of this process. Following the exposure in $^{14}CO_2$ the leaves were removed from the chamber and immediately killed with boiling 96 C ethanol. The fixed material was used for the measurement of the total radioactivity of the leaves, a portion of the material was used for evaluation of metabolic characteristics. Separation of individual substances was achieved with the aid of TLC on cellulose followed with autoradiography. Two-dimensional chromatography was used where the first solvent contained butanol-formic acid-water (6:1:2), and the second one was comprised of propranol-isopropyl alcohol-ammonium hydroxide (1:1:1). Radioactivity was measured by the liquid scintillation method in an Isocap-300 (Nuclear-Chicago). Photosynthetic measurements were carried out in five replicates, estimations of metabolic characteristics in two replicates. The results were highly reproducible; the graphs present the results of individual experiments.

In order to decrease the O_2 content in the leaf chamber it was flushed with helium for 120 sec prior to administration of $^{14}CO_2$. As a result the O_2 content was decreased to 1%. The CO_2 concentration in the majority of the experiments was 0.04%.

Parallel with the photosynthetic measurements the state of stomata was monitored with cellulose acetate replicas taken from the epidermis. Stomata were opened under all conditions of the experiments and, consequently, the specific features of gas exchange reported in the experiments were not related to variations in the stomata opening.

RESULTS

Effect of O_2 on $^{14}CO_2$ Assimilation. With respect to the ratio of the $^{14}CO_2$ assimilation rate at 1% O_2 to the rate of this process at 21% O_2 all of the plant species studied could be divided into three groups. Table I summarizes averaged data obtained for individual species. In the plants of the first group, which comprised all of the C_3 plants, the photosynthetic rate at 1% O_2 is higher than that at 21% O_2 (the Warburg effect). The second group plants include *Haloxylon aphyllum* and *H. persicum*, which display the characteristics of the CAM metabolism. In C_4 plants, photosynthesis could be stimulated by O_2 (the "Warburg antieffect"), *i.e.* it was higher at 21% O_2 compared with that at 1% O_2.

Table I. The oxygen effect of O_2 on $^{14}CO_2$ assimilation at different light intensities

CO_2 concentration was 0.04%. Time of exposure was 1 min. The data are given as cpm/g d.w x 10^{-2}

Type	Species	Light intensity (erg cm^{-2} sec^{-1})					
		80 x 10^3			400 x 10^3		
		O_2 %					
		21	1	1/21	21	1	1/21
C_3	Chenopodium murale L.	180	370	2.1	480	575	1.2
	Lipskuella annua (Winkl.) Juz.	63	114	1.8	136	177	1.3
	Smirnowia turkestana Bunge	70	91	1.3	170	220	1.3
	Heliotropium arguzioides Kar. et Kir.	60	90	1.5	130	169	1.3
	Astragalus paucijugus C.A.Mey	65	84	1.3	150	180	1.2
	Ammodendron conollyi Bunge ex Boiss.	75	98	1.3	140	168	1.2
	Convolvulus divaricatus Rgl. et Schmalh.	70	91	1.3	106	138	1.3
	Senecio subdentatus Ledeb.	105	146	1.3	350	455	1.3
CAM	Haloxylon aphyllum (Minkw) Iljin	25	25	1.0	80	81	1.0
	H. persicum Bunge	25	25	1.0	100	100	1.0
	Salsola richteri (Mog) Kar. ex Litv.	41	41	1.0	70	70	1.0
C_4	Aristida karelinii (Trin. et Rupr.) Roshev.	80	48	0.6	200	140	0.7
	A. pennata Trin.	80	56	0.7	200	140	0.7
	Cynodon dactylon (L.) Pers.	85	59	0.7	-	-	-
	Atriplex dimorphostegia Kar. et Kir.	140	196	1.4	140	108	0.7

The inhibiting effect of O_2 on assimilation of $^{14}CO_2$ in C_3 plant was more significant at low illumination both at limiting and at saturating CO_2 concentration. Maximum values of inhibition were observed at limiting CO_2 concentration. In C_4 plants, the Warburg antieffect could be observed either irrespective of the CO_2 concentration and light intensity (*Aristida karelinii, A. pennata*), or only at full solar irradiation (*Atriplex dimorphostegia*).

The observed possibility of a shift from the inhibiting effect of O_2 to its stimulating influence in *Atriplex dimorphostegia* served as an impetus to investigate this problem in greater detail. Light dependence curves for photosynthesis were obtained at 21% and 1% O_2 (Fig. 1). Under conditions of low illumination (60–120 · 10^3 erg $cm^{-2}sec^{-1}$) photosynthesis is inhibited by O_2, whereas at illumination intensities of above 200 · 10^3 erg $cm^{-2}sec^{-1}$ a stimulation of $^{14}CO_2$ assimilation can be observed as a function of O_2 concentration. Effects of O_2 on photosynthesis were observed not only in C_3, but also in C_4 plants.

Metabolism of ^{14}C in Desert Plants at Natural O_2 Concentrations. Kinetic measurements revealed that the percentage distribution of ^{14}C in some species within a time period of 10 to 90 sec is only slightly changed except for the end products such as sugars.

In *Chenopodium murale* (a C_3 plant) at light intensity limiting photosynthesis about 60% of the assimilated C is represented by the Calvin cycle intermediates. Much of the label is recovered also in malate and aspartate, which contribute above 30% of the radioactivity. An increase in light intensity up to saturation results in an increase in the rate of net $^{14}CO_2$ assimilation approximately 4-fold. The amount of carbon metabolized in the Calvin cycle is also increased, particularly a dramatic increase in radioactivity is observed in sucrose, as well as glucose and fructose. Compared to low illumination, at saturating light intensities the amount of label is increased also in serine and glycine.

In *Smirnowia turkestana*, much like in *Chenopodium murale*, at 80 · 10^3 erg $cm^{-2}sec^{-1}$ the principal carbon assimilated at pulse exposures is concentrated in the Calvin cycle intermediates and alanine. Although the photosynthetic activity of *Smirnowia* is, as a rule, lower than that of *Chenopodium*, this plant at the same exposures features the greater portion of ^{14}C incorporated into the photosynthetic end products sucrose, glucose, and fructose.

At saturating light intensities in *Smirnowia turkestana*, on the whole, the same changes in distribution of ^{14}C occur as those observed in *Chenopodium murale*. This is accompanied with an approximately 5-fold increase in the rate of $^{14}CO_2$ assimilation and an increase in the amount of ^{14}C incorporated into PGA and sugars. As compared to the situation with low light intensities, the concentration of the label is increased also in serine and glycine.

In a representative of the second group—*Haloxylon aphyllum*—the main products of a pulse $^{14}CO_2$ assimilation at limiting light

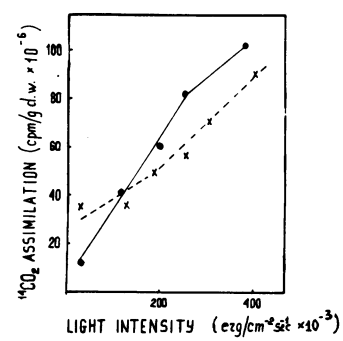

FIG. 1. O_2 effect on $^{14}CO_2$ fixation by *Atriplex dimorphostegia* at different light intensity. (——): 21% O_2; (---): 1% O_2. Pulse of $^{14}CO_2$ increased chamber CO_2 concentration from approximately 0.03 to 0.04%. Specific activity: 1 mCi/l CO_2. Temperature in chamber was 28 to 30 C.

intensities are malate and aspartate. Estimation of the ^{14}C position in malate molecules revealed that 90% of the label is incorporated in the C-4 atom. This indicates that carbon assimilation in this plant, as in typical C_4 plants, proceeds via β-carboxylation. In *Aristida karelinii* (a C_4 plant), similar to the situation with *Haloxylon aphyllum*, the principal metabolites of photosynthesis under low illumination conditions are malate and aspartate. In contrast to *Haloxylon aphyllum*, however, in *Aristida karelinii* the greater portion of ^{14}C is incorporated into aspartate.

In *Atriplex dimorphostegia* (a C_4 plant) aspartate also prevails among the earlier photosynthetic products as is the case for another species of the *Atriplex* genus having the C_4 pathway of metabolism (21).

With an increase in light intensity an increase in $^{14}CO_2$ assimilation could also be observed (2–2.5 times), accompanied by a reduction in the percentage of radioactivity in malate and an increase in alanine. Enhancement of the light intensity does not result in such a sharp rise in the incorporation of ^{14}C into the end products (sugars) in the per cent of the total radioactivity as was the case for C_3 plants at the same exposures.

O_2 Effects on ^{14}C Metabolism in C_3 and C_4 Plants. Primary consideration should be given to the effects of O_2 at various light intensities on the C_3 plant metabolism. Among the C_3 plants studied the inhibiting effect of O_2 on photosynthesis was greatest in *Chenopodium murale*. Reduction of the O_2 concentration to 1% at low illumination increased the net $^{14}CO_2$ assimilation in this plant approximately 2-fold (Fig. 2). This was accompanied by an increase in the radioactivity in all of the compounds analyzed both of the C_3 to C_6 pathway and the C_4 compounds. The highest increase was observed in the radioactivity of PGA, sugar phosphates, and alanine. There is more ^{14}C in serine and glycine at 1% O_2 as compared with 21% O_2.

The Warburg effect is not present at saturating light intensities. O_2 inhibits ^{14}C incorporation into alanine, malate and aspartate and stimulates the ^{14}C incorporation into serine and glycine. No clear-cut effects of O_2 in the Calvin cycle intermediates could be observed (Fig. 3).

Since alanine, malate, and aspartate share a common precursor an attempt was made to elucidate the effects of O_2 concentration on the distribution of the label among these substances in *Chenopodium murale* (Table II). At low light intensities and natural O_2 concentration in the air, the C_4 acid to alanine ratio was 3:6, *i.e.* the synthesis of organic acids prevails over that of alanine (Table II). Reduction in the O_2 concentration down to 1% results in the accumulation of the label in alanine and a resulting decrease in the ratio of radioactivity accumulated in organic acid compared to alanine. Similar evidence concerning a reduced accumulation of oxidized compounds at low O_2 concentrations has been reported for sunflower leaves (8).

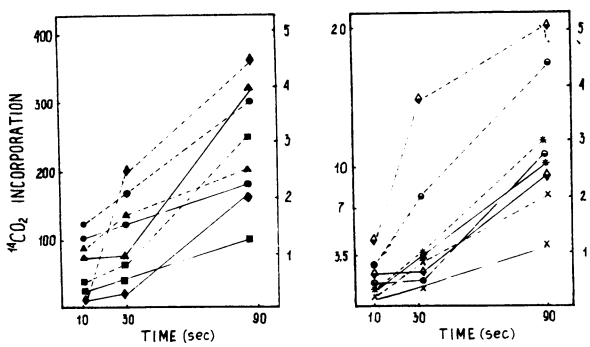

FIG. 2. Effect of O_2 on $^{14}CO_2$ fixation and incorporation of carbon into different compounds by *Chenopodium murale* at $80 \cdot 10^3$ erg cm^{-2} sec^{-1} (in cpm/g dry wt $\times 10^{-6}$). Ordinates on left: total $^{14}CO_2$ fixation (●); sugar phosphates (■); alanine (▼); aspartate (×). Ordinates on right: sucrose (▲); PGA (◆); serine + glycine (◒); malate (*). Other conditions are same as in Figure 1.

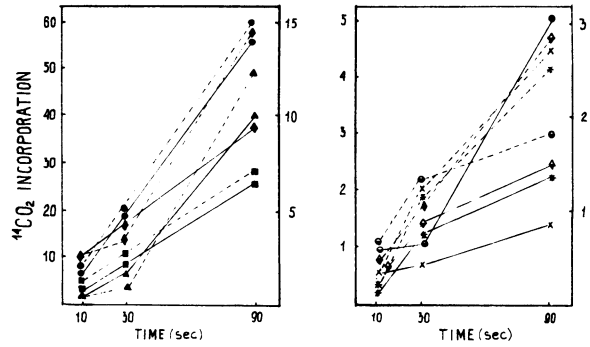

FIG. 3. Effect of O_2 on $^{14}CO_2$ fixation and incorporation of carbon into different compounds by *Chenopodium murale* at $400 \cdot 10^3$ erg cm^{-2} sec^{-1} (in cpm/g dry wt $\times 10^{-7}$) except PGA. PGA (in cpm/g dry wt $\times 10^{-6}$). Ordinates on left: total $^{14}CO_2$ fixation (●); sugar phosphates (■); serine + glycine (◒); aspartate (×). Ordinates on right: sucrose (▲); PGA (◆); alanine (◆); malate (*). Other conditions are same as in Figure 1.

Table II. Incorporation of ^{14}C into alanine, malate and aspartate in Chenopodium murale at various oxygen concentration and light intensity

Exposure in $^{14}CO_2$ (sec)	Oxygen conc.(%)	Malate + aspartate (cpm/g d.w. x 10^{-4})	Alanine (cpm/g d.w. x 10^{-4})	C_4 acids/Alanine
Light intensity $80 \cdot 10^3$ erg $cm^{-2}sec^{-1}$				
10	21	443	145	3.6
30	21	1234	180	6.8
90	21	5195	875	5.9
10	1	735	495	1.5
30	1	2454	1335	1.8
90	1	9710	2490	3.9
Light intensity $400 \cdot 10^3$ erg $cm^{-2}sec^{-1}$				
10	21	415	252	1.7
30	21	1020	875	1.1
90	21	2485	1600	1.3
10	1	768	368	2.1
30	1	2960	1000	2.9
90	1	7250	2690	2.7

At saturating light intensity the incorporation of ^{14}C into alanine increases, whereas that observed for organic acids is only slightly dependent on the light intensity. An increase in the amount of label accumulated in alanine brings about a reduction of the C_4 acid to alanine ratio compared to the situation observed at low light intensities. At 1% O_2 and saturating light intensity the incorporation of ^{14}C in organic acids is increased compared to the incorporation into alanine.

In *Smirnowia turkestana* the response of metabolism to changes in O_2 concentration is similar to that reported for *Chenopodium murale*: the most notable response was recorded in PGA, sugar monophosphates, and alanine. The inhibiting effect of O_2 in *Smirnowia* is less pronounced. At 21% O_2 we observed a somewhat elevated incorporation of ^{14}C into glycine and serine compared to that at 1% O_2.

At light intensity saturating photosynthesis the inhibiting effect of O_2 upon the ^{14}C assimilation in *S. turkestana* is only slightly manifested. Differences in the accumulated radioactivity are maintained only for alanine; at 1% O_2 its radioactivity is found to be 40 to 60% higher than that at 21% O_2. In contrast to *Chenopodium*, *Smirnowia* we observed a slight stimulation of accumulation of ^{14}C in sucrose at elevated O_2 concentrations.

In a representative of another group of plants—*Haloxylon aphyllum*—no sensitivity to O_2 concentration could be detected based on the net $^{14}CO_2$ assimilation. Nonetheless, notable changes in the accumulation of the label can be traced in some compounds (Fig. 4). These changes amount to a suppression of the ^{14}C incorporation into malate and aspartate at 1% O_2, and, conversely,

the incorporation into the Calvin cycle metabolites, as well as starch is stimulated as is the case with typical C_3 plants.

Next the effects of O_2 on the metabolism of C_4 plants will be considered.

In *Aristida karelinii* at low illumination and 1% O_2 the net $^{14}CO_2$ assimilation is decreased compared to that at 21% O_2, *i.e.* an effect opposite to the Warburg effect is observed (Fig. 5). This reduction of the net $^{14}CO_2$ assimilation at 1% O_2 is accompanied by an inhibition of the incorporation of the label into malate and aspartate as well as glycine and serine. Regarding C_3 and C_6 compounds, at 1% O_2 in *Aristida karelinii* we observed the inhibiting O_2 effect on the biosynthesis of sugar phosphates, and alanine, whereas with prolongation of the exposure the effect extends also to the biosynthesis of sucrose and starch (typical of C_3 plants). This plant shows a dramatic increase in radioactivity in serine and glycine at 21% O_2.

Another dependence of carbon metabolism upon the O_2 concentration was detected in *Atriplex dimorphostegia*—another C_4 plant. With a decrease in the O_2 concentration in the air at low illumination this plant displays changes in the distribution of the label among metabolites which are analogous to those found in C_3 plants (Fig. 6). the photosynthetic intensity at 1% O_2 was higher than at 21% O_2. At 1% O_2 the incorporation of labeled carbon into sugar phosphates, alanine, and malate was stimulated. A similar situation could be observed with O_2 effects on *Chenopodium murale* (Chenopodiaceae) which belongs, however, to C_3 plants. Reports on the possible changes in the metabolism of ^{14}C as affected by O_2 concentration in *Zea mays* (similar to C_3 plants) may be found in the literature (15).

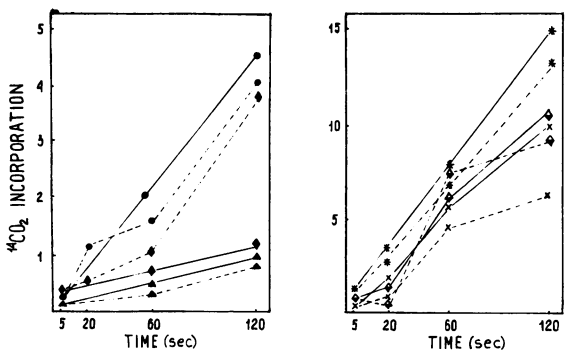

FIG. 4. Effect of O_2 on $^{14}CO_2$ fixation and incorporation of carbon into different compounds by *Haloxylon aphyllum* at $80 \cdot 10^3$ erg cm^{-2} sec^{-1} (in cpm/g dry wt $\times 10^{-5}$; total $^{14}CO_2$ fixation and alanine—in cpm/g dry wt $\times 10^{-4}$). Total $^{14}CO_2$ fixation (●); sucrose (▲); PGA + sugar phosphates (◆); aspartate (×); malate (*); alanine (⬙). Other conditions are same as in Figure 1.

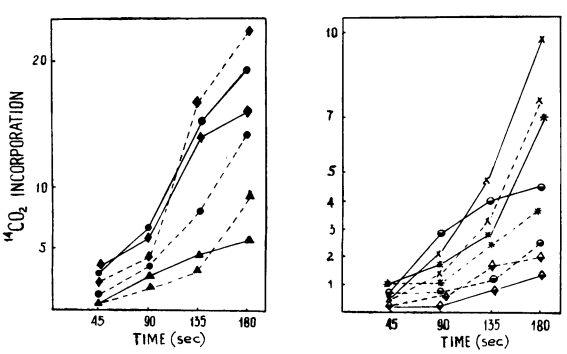

FIG. 5. Effect of O_2 on $^{14}CO_2$ fixation and incorporation of carbon into different compounds by *Aristida karelinii* at $80 \cdot 10^3$ erg cm^{-2} sec^{-1}. (Total $^{14}CO_2$ fixation, aspartate, alanine, malate: in cpm/g dry wt $\times 10^{-6}$; PGA + sugar phosphates, serine + glycine: in cpm/g dry wt $\times 10^{-5}$; sucrose: in cpm/g dry wt $\times 10^{-4}$). Total $^{14}CO_2$ fixation (●); sucrose (▲); PGA + sugar phosphates (◆); serine + glycine (⊖); aspartate (×); alanine (⬙); malate (*). Other conditions are same as in Figure 1.

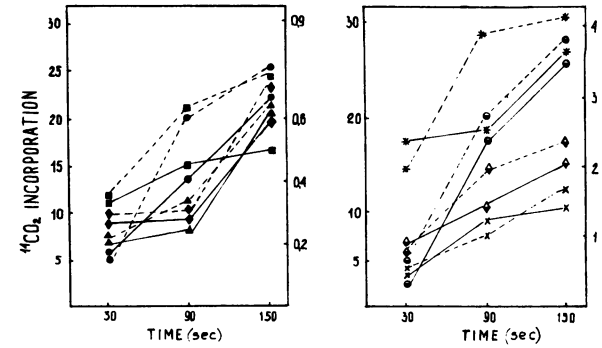

FIG. 6. Effect of O_2 on $^{14}CO_2$ fixation and incorporation of carbon into different compounds by *Atriplex dimorphostegia* at $80 \cdot 10^3$ erg cm^{-2} sec^{-1}. (In cpm/g dry wt $\times 10^{-6}$; sugar phosphates: in cpm/g dry wt $\times 10^{-5}$; serine + glycine: in cpm/g dry wt $\times 10^{-4}$). Ordinates on left: total $^{14}CO_2$ fixation (●); sugar phosphates (■); serine + glycine (⊖); aspartate (×). Ordinates on right: sucrose (▲); PGA (◆); alanine (⬙); malate (*). Other conditions are same as in Figure 1.

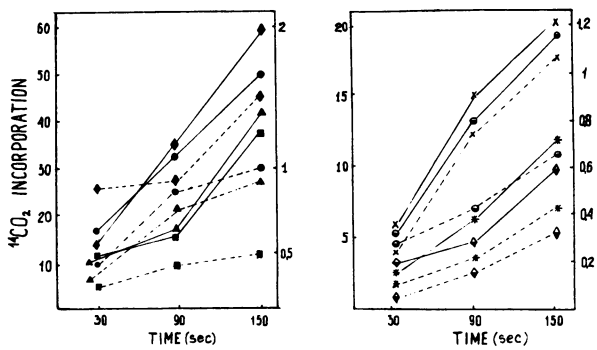

FIG. 7. Effect of O_2 on $^{14}CO_2$ fixation and incorporation of carbon into different compounds by *Atriplex dimorphostegia* at $400 \cdot 10^3$ erg cm^{-2} sec^{-1}. (In cpm/g dry wt $\times 10^{-6}$; sugar phosphates in cpm/g dry wt $\times 10^{-5}$). Ordinates on left: total $^{14}CO_2$ fixation (●); sugar phosphates (■); aspartate (×); malate (*). Ordinates on right: sucrose (▲); PGA (◆); serine + glycine (⊖); alanine (⬙). Other conditions are same as in Figure 1.

At saturating light intensities in *Atriplex dimorphostegia* the direction of the photosynthetic effect by O_2 is reversed. At 1% O_2 a decrease in the net $^{14}CO_2$ assimilation could be observed compared with that at 21% O_2 (Fig. 7), *i.e.* there occurs the Warburg antieffect, the one reported earlier for *Aristida*.

A reduction in the O_2 concentration results in inhibition of the incorporation of ^{14}C into malate (approximately 1.8 times compared to the situation at 21% O_2). Since most of the label is incorporated into malate and aspartate, the reduction in the net $^{14}CO_2$ assimilation at 1% O_2 is primarily correlated with a decrease in the accumulation of ^{14}C in these compounds. The more pronounced changes are observed in the radioactivity of malate.

In *Atriplex dimorphostegia* we observed an inhibition of the ^{14}C incorporation into the three- and six-carbon atom compounds at 1% O_2, most notably in sugar phosphates and alanine.

DISCUSSION

Among the plants studied all of the C_3 species displayed the usual effect of inhibition of photosynthesis at natural O_2 concentrations. The enhancement in the rate of assimilation at 1% compared with 21% O_2 is, as a rule, 30 to 50% consistent with the evidence found in the literature (3, 36). An analysis of the conditions under which the inhibiting action of O_2 is most pronounced has revealed that the maximum values of the Warburg effect are obtained at low illumination and low CO_2 concentration. The highest magnitude of the Warburg effect was found in *Chenopodium murale*

A group of plants (CAM?) in which the photosynthetic activity is not changed with the O_2 concentration is of great interest. This group comprises plants having either cylindrical leaves (*Salsola richteri*), or aphyllous species (*Haloxylon aphyllum* and *H. persicum*) showing succulent characteristics.

In typical C_4 plants, we observed a stimulating effect of O_2 on the $^{14}CO_2$ assimilation. This phenomenon has also been observed with prolonged exposures (about 10 min). Of the greatest interest is the C_4 plant *Atriplex dimorphostegia* in which both the inhibiting O_2 effects on photosynthesis (at low light intensity) and its stimulating effect (at saturating light intensity) could be observed.

As a rule, inhibition of photosynthesis by O_2 is related to enhancement in the glycolate pathway metabolism (4, 6, 10, 14, 17). The findings reported in this study indicate that the Warburg effect is not only involved with the glycolate pathway. Thus, in the C_3 plants (*Chenopodium murale* and *Smirnowia turkestana*) the Warburg effect is most clear-cut under conditions of limiting light intensities, whereas the per cent of radioactivity in glycine and serine is, on the contrary, more abundant at light intensities saturating for photosynthesis. Besides, in *Chenopodium murale* in which the O_2 inhibition of photosynthesis was most pronounced, no correlation could be detected between the accumulation of ^{14}C in serine and glycine and the O_2 concentration in the environment.

On the other hand, in a typical representative of the legume plants (*Smirnowia turkestana*) there is a tendency for an elevated incorporation of ^{14}C into serine and glycine at 21% O_2 compared to that at 1% O_2. The presence of the glycolate pathway in this plant, however, is insufficient to account for the inhibiting effect of O_2 on the net gas exchange in every detail.

Differences in carbon incorporation in different compounds in C_3 plants as a function of O_2 concentration provide the following conclusions. Since the main portion of ^{14}C is incorporated into the Calvin cycle intermediates (primarily into the sugar phosphates), the inhibition of the uptake of carbon by O_2 into these compounds determines the general suppression of the $^{14}CO_2$ assimilation, observed under conditions when light intensity limits photosynthesis. The lesser incorporation of ^{14}C into sugar phosphates at 21% O_2 compared to that at 1% O_2 was reported also for sunflower leaves (1).

Irrespective of the plant species, at high O_2 concentrations the incorporation of ^{14}C into alanine is inhibited. Similar evidence concerning the dependence of alanine formation on O_2 concentration has been reported for kidney beans (22, 25). Apparently those conditions which inhibit the processing of carbon through the Calvin cycle (low illumination, an elevated O_2 concentration) also serve to retard the incorporation of ^{14}C into alanine. Earlier it had been demonstrated (13) that the accumulation of the label in this amino acid in *Chlorella* is related to the functioning of PSII.

In *Chenopodium murale*, in which organic acids are important in the metabolism, the biosynthesis of not only three-carbon, but also of four-carbon compounds is inhibited by O_2. As a result, the over-all effect of its action on photosynthesis proves to be maximum in *Chenopodium murale* as compared with other C_3 plants.

In another C_3 plant (*Smirnowia turkestana*) the principal regularities of changes in metabolism affected by variations in the O_2 concentration appear to be essentially the same as in *Chenopodium murale*. A characteristic feature of the photosynthetic metabolism in *Smirnowia*, compared to *Chenopodium murale*, is the more rapid processing of carbon to the end products (sugars). The formation of these compounds (similar to the situation in *Chenopodium murale*) proves to be less sensitive to O_2 concentration. The independence of the rate of sucrose biosynthesis of the O_2 concentration has also been reported in kidney bean (26). This phenomenon is explained in the literature as follows: it is assumed that at high O_2 concentrations the formation of sucrose is enhanced not via the triose phosphates but rather through the intermediates of the glycolate pathway (2). The synthesis of sucrose in an alternative way (via the intermediates of the glycolate pathway) assumes, however, localization of this process in the cytoplasm. At the same time, there is evidence available that glycine and serine (putative precursors in the synthesis of sucrose) are located both in C_3 and C_4 plants not only in the cytoplasm, but also in chloroplasts (7, 18).

The changes in the rate of formation of metabolites described above as a result of carboxylation of ribulose-diP can be shown only at the light intensities limiting photosynthesis. At saturating light intensity the incorporation of $^{14}CO_2$ into sugars is dramatically increased.

With an increase in the light intensity from 80 to $400 \cdot 10^3$ erg $cm^{-2}sec^{-1}$ the rate of $^{14}CO_2$ assimilation increases and a similar effect is produced by a reduction in the O_2 concentration, it was then natural to investigate whether the limitation of light could in any extent be compensated for by a reduction in O_2 concentration in the air. The experiments reported in this paper (Table III) revealed that such an interchangeability is indeed probable. Thus, in *Chenopodium murale* at low light intensity one can obtain, by lowering the O_2 concentration, the incorporation of ^{14}C into sugar phosphates and PGA roughly corresponding to the respective values in the experiments with saturating light intensities and 21% O_2. Notable differences are, however, still observed in the incorporation of ^{14}C into sucrose.

Our results are consistent with the ideas of O_2 inhibition of ribulose-diP carboxylase, triose-P dehydrogenase, and phosphoribulokinase (5, 10). Some of these enzymes are light-activated (19, 37).

In our opinion, the oxygenase activity of ribulose-diP carboxylase is not crucial in determining the over-all effect of O_2 on photosynthesis.

One of the main conclusions of the present study is that carbon metabolism not only in C_3, but also in C_4 plants, is dependent on the O_2 concentration. In contrast to C_3 plants in which O_2 inhibits the $^{14}CO_2$ assimilation, we observed a stimulating effect of O_2 on this process in the C_4 plants studied.

Evidence of the possibility of the stimulation by O_2 of the $^{14}CO_2$ in C_4 plants was previously described in a study by Huber and Edwards (16) with *Digitaria* protoplasts.

It is conceivable that differences in the structural organization of C_3 and C_4 plants are responsible for the distinct pattern of the dependence of the photosynthetic metabolism upon the O_2 concentration. The dependence of the net photosynthetic gas exchange in C_4 plants on O_2 is large and is explained by the relationships between the operation of two cycles: (*a*) carboxyla-

Table III. Effect of oxygen at various light intensities upon the distribution of ^{14}C among metabolites in Chenopodium murale (exposure 30 sec)

Variants		Radioactivity of individual compounds (cpm/g d.w. x 10^{-4})									
Oxygen concentration (%)	Light intensity (erg $cm^{-2}sec^{-1}$ x 10^{-3})	Total	PGA	Sugar: Monophosphates	Sugar: Diphosphates	Sucrose	Glucose + fructose	Alanine	Malate	Aspartate	Serine + glycine
21	80	4170	29	1570	587	29		180	630	600	158
1	80	12800	240	5480	1000	100		1300	880	1570	205
21	400	17900	320	6800	3200	730	260	870	500	520	2156
1	400	20400	264	6320	4080	1060	264	1000	1230	1730	1386

tion of P-enolpyruvate resulting in the synthesis of malate and aspartate and their subsequent decarboxylation; and (*b*) the Calvin cycle itself. An example of a plant in which the inhibiting and stimulating O_2 effects on individual stages of the photosynthetic metabolism are well balanced may be seen in *Haloxylon aphyllum*, the plant in which, according to Vosnesenskaya (28), the second lower layer of chlorenchyma acts as bundle sheath cells. In this plant no changes in the over-all gas exchange are observed as a function of the O_2 concentration neither at limiting, nor at saturating light intensities.

The larger accumulation of ^{14}C in malate and aspartate with elevation of O_2 concentration from 21 to 100% has been also reported for maize leaves (20). That finding was related not to the higher rate of synthesis of C_4 acids at elevated O_2 concentrations, but rather to the lower rate of transfer of ^{14}C from dicarbonic acids to the Calvin cycle intermediates.

No satisfactory explanation of the fact that in C_4 plants the incorporation of ^{14}C in dicarboxylic acids is stimulated by O_2, whereas in C_3 plants, it is inhibited, can be offered at this time. These differences are apparently related to the requirements of intertissue transport of C_4 compounds to the bundle sheath cells and to the probable participation in the transport processes in respiration.

As concerns the bundle sheath cells in C_4 plants, there occurs in these an inhibition of those same links in metabolism as in typical C_3 plants. The dependence of the net gas exchange on O_2 in C_4 plants is then determined by specific ecological conditions. Thus, in *Atriplex dimorphostegia* at low light intensities, O_2 inhibits $^{14}CO_2$ assimilation while at saturating light it stimulates this process. The O_2-sensitive site appears to be primarily the biosynthesis of malate.

An effect of O_2 on photosynthesis in C_4 plants may be in the various stages of regeneration of the CO_2 acceptor. The regeneration of the acceptor may be related to the intensity of the dark respiration, which, in its turn, may be dependent on the O_2 concentration. In the light, the dark respiration may contribute to the over-all metabolism in such a complex and heterogeneous system as is found in the leaf.

In conclusion it should be emphasized that the dependence of the rate of $^{14}CO_2$ incorporation on O_2 concentration in C_3 and C_4 plants observed with pulse exposures persists also with prolongation of the exposure up to 10 min.

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